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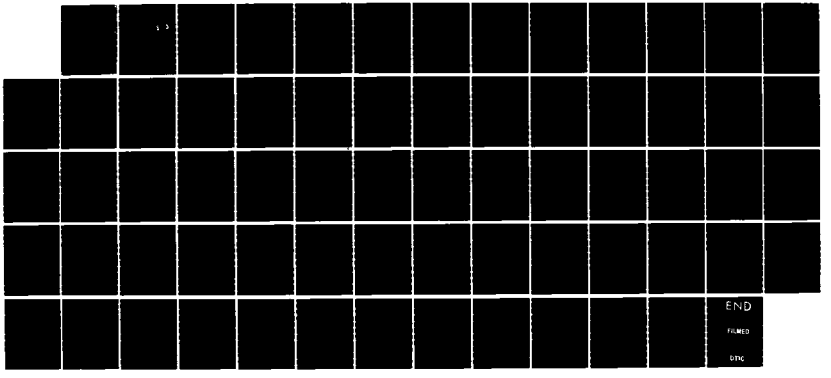

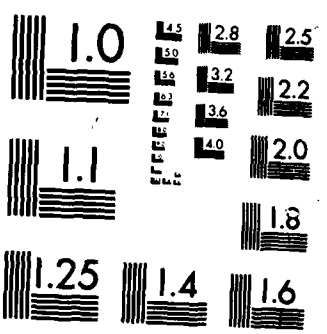

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ANALOG MEMBRANE DISINFECTION INDICATOR AND HALOGEN ELECTRODE

Final Report

J. Donald Johnson, Ph.D.

May 1983

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

Contract No. DAMD17-76-C-6058

University of North Carolina
Chapel Hill, North Carolina 27514

David H. Rosenblatt, Ph.D., Contracting Officer's Technical Representative
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The proof of concept of the Analog Membrane Disinfection Indicator, AMDI, was completed as a rapid simple integrating measuring system for the extent of chlorine disinfection of water supplies. A methyl red dyed membrane strip was able to integrate the total concentration-time response of chlorine by comparison with the chlorine electrode and also in a time response analogous to the disinfection of the spore of B. subtilis. A neutral red dyed AMDI was more sensitive and less subject to high pH interference than the methyl red AMDI

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Summary

A new method for measuring the effectiveness of chlorination by the integration of the total effect of changing concentration with time on water disinfection has been developed as far as the proof of the concept and the production in the laboratory of a test system. The analog membrane disinfection indicator, AMDI, is a simple colorimetric method of measuring disinfection efficiency during a water disinfection process with chlorine. During this process, the concentration of chlorine decreases rapidly, and the results in this study show the concentration at the end of the disinfection process, commonly measured and referred to as residual chlorine, does not define the effectiveness of disinfection or safety of the water for drinking. Disinfection is not a function of residual chlorine, but of the total integration of changing chlorine concentration with time. The AMDI was designed to measure this total integral. In addition, through the use of the membrane separation process, developed under a previous Army Medical Research & Development Command contract, the ineffective for disinfection hypochlorite form of chlorine is excluded from the measurement.

This research has provided a demonstration of the principles of the analog membrane disinfection indicator, AMDI, and has shown the feasability of the concept by developing a plastic film, dyed with a colorimetric indicator for chlorine. The colored dye is bleached as chlorine dissolves or diffuses into the film as a function of time and the concentration of the effective for disinfection form of chlorine, hypochlorous acid. The color disappeared as a first order function of time, analogous to the first order dependence of organism survival with time. This analogous behavior of the measurement was tested versus the disinfection of the difficult to kill B. subtilis bacterium and shown to correlate with the level of disinfection, while the usual method of measurement of residual chlorine did not.

The AMDI strip is a neutral red dyed ion exchange film. A procedure has been developed to dye the film, which gives reasonable stability and sensitivity to chlorine. The physical properties of this film have been measured with respect to temperature, dye, and pH stability.

Although the AMDI appears to be a promising measurement system, it requires further characterization and development. It needs to be tested as a function of pH, ammonia, ionic strength, temperature and wider concentration range. Finally, to aid in the visual determination of a color end point for the level of disinfection required, a color masking agent could be incorporated to change a level of color in the film into a change in contrasting color.

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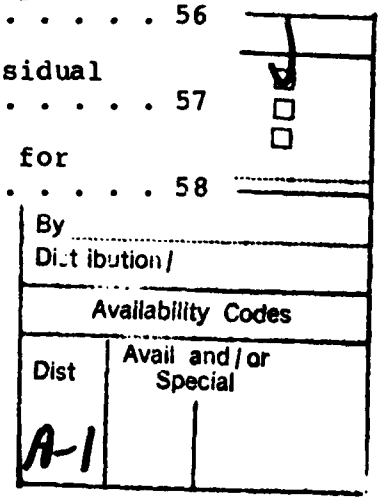

I. Introduction

Current methods for controlling disinfection processes typically rely on a single measurement of a residual free chlorine concentration after a particular contact time. Such a measurement only determines the concentration of chlorine remaining in the solution at that single point in time. Because chlorine is a strong oxidizing agent, its concentration rapidly decreases with time. The efficiency of the disinfection process in practical systems is strongly dependent upon changes in chlorine concentration that usually occur during the contact period. Rather than the residual concentration, it is the integration of changing disinfectant concentration as a function of time, termed the germicidal (or microbiological) dose,[1] that actually provides a measure of the degree of kill of microorganisms which has been achieved.

In addition to changing concentration and contact time, disinfection efficiency also depends upon the distribution of the available chlorine among its various chemical forms: molecular (Cl_2), hypochlorous acid (HOCl), hypochlorite ion (OCl^-), and chloramines (NH_2Cl, $NHCl_2$, NCl_3, and organic NCl). These forms differ considerably in their strength as disinfectants for various pathogens.[1] A serious shortcoming of most current tests for free available chlorine is the inability to directly differentiate between the two principal chlorine forms, HOCl and OCl^-. The distribution among the various forms and the overall disinfection efficiency, even at constant residual concentration, is a strong function of pH. However, the disinfection efficiency of any one chemical form is relatively independent of pH, except at extreme values. The same can be said for disinfection efficiency varying with ammonia concentration. Both chlorine chemical forms and concentrations vary simultaneously in typical practical systems as hydrolysis, amination, and redox reactions

occur as a function of time. This change in chlorine chemistry generally results in a decrease in the effectiveness of chlorine as a disinfectant with time.

The rate of kill of microorganisms is also directly dependent upon temperature. This important factor is often not considered in setting residuals required for disinfecting field water supplies. All of these effects can significantly alter the disinfection efficiency for a given chlorine dose. Yet, few if any of these effects are taken into account when, as is typically done, only a single residual chlorine measurement is used to determine whether a drinking water has been disinfected.

The overall objective of this research project was to develop a disinfection indicator system that would provide a simple colorimetric method we refer to as the analog membrane disinfection indicator, AMDI, for more reliably evaluating the disinfection efficiency of chlorine, using all of the major variables discussed above. The color change of this indicator system, which consists of a dye incorporated within a plastic strip, would be proportional to the level of disinfection attained, not just to residual concentration. The visual end point of the system would provide a "go-no-go" indication of whether the disinfection process had been adequate to produce safe water. This visual end point could also be varied or matched so as to correspond to a desired level of kill for different types of pathogens, depending on the hazards present in a given geographical area.

To achieve this long-term objective of measuring disinfection, the system must integrate, as a function of time, the changing concentrations of various chemical species of the disinfectants that form and disappear during the disinfection process. Ideally, the reading of this concentration-time

integral or disinfectant germicidal dose would also be proportional to the disinfection efficiency of each chemical species of disinfectant forming and decomposing as a function of time. In addition, the color change of the disinfection indicator system should depend on temperature in the same way the disinfection process depends on temperature. A disinfection indicator system of this sort would then integrate varying concentration as a function of time; furthermore, it would read each of these integrals as a number proportional to the disinfection efficiency of the chemical species whose concentration was being integrated and at a rate dependent on temperature in the same general way that the disinfection process depends on temperature.

A. Background

The vital genetic and enzymatic constituents of microorganisms are generally shielded from the attack of chemical disinfectants such as chlorine by a surrounding cell membrane, protein coat, cell wall, and/or slime film. The disinfection process then consists of two steps. The first step is the transport of the disinfectant from the external solution to sensitive sites within the microorganism. The second step involves, in the case of chlorine, the oxidation of a genetic, enzymatic, or other sensitive constituent that is biochemically important. Eventually, damage is done to the extent that the cell can no longer function, and disinfection or inactivation has occurred.

In a classic paper in 1948, Fair et al.[2] showed that the relative efficiency of disinfection with HOCl was approximately 100 times that of OCl^-. They also showed that the temperature dependence of the disinfection action of aqueous chlorine was low compared to that of chemical processes. Chemical processes

normally have a Q_{10} (increase in rate for a 10^{o} C rise in temperature) of two or more, while the Q_{10} of aqueous hypochlorous acid disinfection was from 1.4 to 1.65. In general, processes that are dependent on the rate of diffusion or other mass transport processes have relatively low Q_{10} values, while chemically controlled processes often have Q_{10} values of two or more. This suggests that the rate limiting step in disinfection by HOCl depends upon the rate at which the HOCl can be transported to an active site within the microorganism, or that the slowest step in the disinfection process is the rate of transport of HOCl through the cell membrane or other physical barrier. It is logical then that OCl^-, a larger and more polar species, would have more difficulty in getting through the cell membrane to reach an active site. This explains why OCl^- is a much poorer disinfectant than HOCl, although it may be equally effective as an oxidant at a given pH.

The development of an HOCl membrane electrode under the direction of this principal investigator, with the U.S. Army Medical R & D support, has permitted the selective measurement of HOCl in the presence of OCl^-.[3] This membrane electrode has provided an important new analytical capability for monitoring disinfection processes, and was also an important step toward the development of an analog membrane disinfection indicator (AMDI).[4] The membrane electrode has been utilized in a recent research paper in which it has been demonstrated that microbiological dose does serve as a measure of disinfection. The destruction of B. subtilis spores by HOCl was found to be correlated to the microbiological dose under conditions of both constant and changing (due to demand) HOCl concentrations.[5]

Ongoing research under this contract on an analog membrane disinfection indicator has resulted in the development of a trial system consisting of neutral red dye adsorbed within a Nafion

(trade name, E.I. DuPont Co.) cation exchange, polyfluoroethylene copolymer membrane. Various properties and modifications of this system have been investigated. The color change (bleaching) of this system has been found to follow first order kinetics, analogous to the disinfection process. Selective response for HOCl over OCl^- and chloramines has also been observed.

B. Research Goals

The overall goal of this research is the development of an Analog Membrane Disinfection Indicator, AMDI, the response of which will parallel the actual disinfection experienced by microorganisms in a given water supply upon the addition of chlorine. To do this, the AMDI system must integrate the changing concentration of disinfectant as a function of time, while also taking into account the effects of temperature and changes in chlorine speciation due to pH or ammonia. The AMDI concept is important because disinfectant concentrations and chemical characteristics change rapidly during the disinfection process. The importance of changing concentration, temperature, pH, and ammonia effects makes interpretation of current residual concentration measurements in terms of disinfection efficiency difficult at best.

C. Hypotheses

The general concept of this research is that a plastic membrane, analogous to a biological membrane, will limit the rate of mass transport of the disinfectant chemical species into the indicator system, thereby resulting in a reading proportional to the relative effectiveness of the disinfection process. The color change of the indicator system will thus provide a measure of the integration of disinfectant concentration as a function of time. In addition, the response of the system to different

chlorine species will correspond to their relative disinfection efficiencies. The measurement by the disinfection indicator will also be dependent on temperature in the same manner as the mass transport of disinfectant in a cell membrane. Thus, the temperature dependence of the measurement will be similar to that of disinfection, since the rate of mass transport is the limiting step in both processes.

By placing an analog membrane disinfection indicator, AMDI, into a batch of water at the same time that the disinfectant is added, the indicator will experience the same environmental conditions as the pathogenic organisms in the water during the disinfection process. Ideally, the AMDI measurement will then incorporate the effects of changing chemical species and concentrations, pH, temperature and duration of treatment, in a manner consistent with the influences of these variables on the disinfection efficiency. Such an indicator will thus provide a simple, yet highly reliable method of insuring that treated water is safe for use.

In general, the rate of kill of a microorganism is related to time and disinfectant concentrations as follows:

$$\ln \frac{N}{N_o} = -\Lambda D = -\Lambda \int_{t_o}^{t} C^n dt \qquad (1)$$

where, ln = the natural logarithm (base e)
N = number or organisms
N_o = initial number of organisms
Λ = specific lethality coefficient
D = germicidal dose
t = time
C = concentration of disinfectant (which can vary with time)
n = coefficient of dilution (generally assumed to be one)

For the color change of the indicator to be proportional to the level of disinfection attained, the bleaching kinetics should be first-order and should conform to the following relationships:

$$\ln \frac{A}{A_o} = K \ln \frac{N}{N_o} = -K \Lambda D \qquad (2)$$

where, A = absorbance of the indicator strip

A_o = initial absorbance of the indicator strip

K = a proportionality constant

II. Experimental

A. Materials

The final AMDI system developed in this study consists of neutral red dye incorporated within a nafion cation exchange membrane. In this system, the rate of bleaching of neutral red by chlorine (or hypochlorous acid) is limited by diffusion of the chlorine into the membrane. Also, the presence of negatively charged sites within the Nafion membrane inhibits the diffusion of OCl^- into the system.

Neutral red is an azine dye that is readily oxidized by free available chlorine, but is only slowly oxidized by chloramines. It is incorporated within the Nafion film by absorption from aqueous solution. The resulting indicator film is red in color.

The Nafion cation exchange membrane is a clear perfluorosulfonic acid product of DuPont. The Nafion membranes in use have an equivalent weight of 1100, and thicknesses of 4 or 10 mils. The water content of these membranes was altered in various ways in order to modify the performance of the system.

B. subtilis spores were produced in a stock suspension of 5 x 10^7 spores/mL. This suspension was microscopically examined periodically to insure that only aggregate-free spores would be present. Spores were counted by standard serial dilution and plating techniques.[6]

Reagents, polarographs, laboratory equipment, and the HOCl membrane electrode used in this research are the same as those described earlier for this laboratory.

B. Methods

1. Spectrophotometric Measurements of AMDI Strips

Absorbances of dyed membrane strips were measured with a Cary 14 recording spectrophotometer or a Beckman DB spectrophotometer with 1 cm cells containing chlorine demand free water (CDFW). The strip was held in place against the inside of the sample cell by a special plastic block that had been drilled out in the middle along the light path. Correction was made for the absorption of the undyed membrane by subtracting out the absorbance measured for undyed strips from the observed absorbance of the dyed strip.

2. Determining Water Absorption of Membranes

Strips of membrane were weighed to the nearest 0.0001 gram using a Mettler "Gram-atic" Balance. Wet membranes were quickly blotted between sheets of Whatman No. 1 filter paper to remove surface water, and then were weighed as quickly as possible to avoid evaporation losses. This blotting technique has been previously used by others,[7] and highly reproducible results were obtainable in this manner. The strips of membrane were usually allowed to equilibrate in water for at least 10 h prior to

weighing, and the wet strips were then allowed to air-dry for at least 10 h before dry weights at room temperature and humidity were measured. Absolute dry weights were measured after drying in an oven at 105° C for at least 2 h and then desiccating. The times noted above were found sufficient for constant weights to be reached. Absolute dry weights have been estimated in many cases by correcting the air-dry weights for the amount of water retained at room temperature and humidity, as approximated from other tests and from data in the literature.[8,9] Water absorptions determined from such estimated dry weights have been reported as approximate values.

Measurements of dry and wet dimensions were performed with strips that had been handled as described above. Length and width were measured with a metric ruler, and a standardized paper micrometer was used to measure thickness.

3. Determination of Dye Sorption

A strip of Nafion membrane, for which the weight and water absorption had been measured, was immersed into an aqueous dye solution of known concentration and volume. The dyeing was continued until the measured absorption, λ_{max}, of the strip reached the desired level; the strip was then removed, and its final absorption was recorded. The remaining concentration of dye in the solution was determined spectrophotometrically according to a standard curve prepared with dye solutions of known concentration. The quantity of dye sorbed was then calculated from the difference between the initial and final concentrations, along with the known volume of the solution.

4. Bleaching

During testing of the system, the color intensity of indicator strips was measured spectrophotometrically at the

visible wavelength of maximum absorbance. Investigations of bleaching of the indicator were conducted in chlorine solutions buffered to suitable pH values and at constant temperature. Experiments were typically performed at 20° C. The chlorine solutions were continuously stirred and at similar ionic strength to further standardize conditions. Constant chlorine concentrations were determined by amperometric titrations, while changing chlorine concentrations under chlorine demand conditions were measured with the HOCl membrane electrode. Demand conditions were simulated by the slow addition of thiosulfate.

The bleaching kinetics of the indicator were investigated as a function of dose by measuring color change with time under conditions of various constant and changing chlorine concentrations. Then, testing of bleaching kinetics in conjunction with the measurement of kill of microorganisms was performed with disinfection resistant spores. These disinfection experiments were performed under various conditions, as described above, to allow for direct evaluation of the parallel between the performance of the AMDI and the disinfection process.

5. Bleaching of AMDI Compared to Spore Inactivation

Spore experiments were of two types. One type consisted of experiments at constant concentration. The second type consisted of experiments in which the concentration of chlorine was purposefully changed by use of $Na_2S_2O_3$ as demand. All calibrations and experiments were run at pH 7 and 30° C.

After the HOCl membrane electrode was calibrated, the initial concentration for the run was chosen and chlorine was added to achieve this concentration in 200 mL of buffered chlorine demand free water. After the electrode response had stabilized, 2 mL of stock spore suspension were added to the stirring solution.

After 1 minute, to allow mixing, the first sample, at time t_o, was withdrawn. A total of 7 or 8 samples were withdrawn during an estimated germicidal chlorine dose of 30 ppm minutes. If $Na_2S_2O_3$ was added, the rate of addition was adjusted before the experiment began and its addition started after t_o.

Concentrations of viable spores were reported as spores/mL and finally as $\log_{10} N/N_o$. The chlorine concentration was recorded as microamperes versus minutes and integrated by counting the squares. This method of calculating D is valid only if n = 1 in Equation 2. Cumulative microbiological dose was figured for all sample times. The resulting D and $\log_{10} N/N_o$ were plotted and a best fit line was drawn through the straight line portion by linear regression. The slope of this line yielded $-\Lambda_{10}$. The lag period was excluded from the linear regression as was any obvious tailing at the end of the experiment.

III. Results and Discussion

A. Preparation of the AMDI

1. Initial Selection and Preparation

Various possibilities for incorporation of a chlorine indicator into a plastic membrane were initially considered, including: enclosure of indicator within a membrane pillow, micro-encapsulation, ion exchange, and bonding within the membrane structure during polymerization. Early investigations indicated the feasibility of the ion exchange approach, by use of DuPont Nafion membranes, which are structurally similar to the DuPont XR-280 microporous membranes studied for use with the halogen membrane electrode.[3] Films that were prepared by Dr. Harry Gregor, Columbia University, with incorporation of

indicators during polymerization were found to be unsatisfactory, since they were hydrophobic and insensitive to chlorine. While other approaches may be useful in future development of the AMDI system, the ion exchange approach has been employed in this stage of our research, inasmuch as the Nafion system showed potential for development without the need for further efforts in fabrication of membranes.

The properties of Nafion membranes make them highly suitable for use in the AMDI system. Nafion is DuPont's trade name for its perfluorosulfonic acid polymer, which is available as clear, homogeneous membranes of various thicknesses and equivalent weights. Our investigations have included the use of Nafion 110 (1100 equivalent weight, 10 mils thickness) and Nafion 114 (1100 equivalent weight, 4 mils thickness). As a cation exchange membrane, Nafion allows the incorporation of a cationic chlorine indicator by ion exchange. The strongly ionic nature of Nafion membranes imparts to the system the desired selectivity for HOCl by inhibiting diffusion of OCl^-, which is a much weaker disinfectant than HOCl. Apart from the sulfonic acid groups, the fluorocarbon composition of the polymer makes it quite chemically inert, and provides good stability to oxidants, including chlorine. Nafion membranes are very hydrophilic, with rapid water absorption and the capacity for a high water content, thereby making them suitable for use in aqueous systems. The transparency of the membranes permits spectrophotometric measurements of the incorporated indicator.

Methyl red was initially selected for use with Nafion membranes, since it is an indicator that is bleached by free chlorine with low interference from combined chlorine.[10] Methyl red is also structurally similar to methyl orange, an indicator that has been frequently used for measuring chlorine.[11] In initial testing, Nafion membranes were successfully dyed with

methyl red, and the colored film was then gradually bleached in the presence of HOCl. A possible alternative to the use of an indicator that is bleached by chlorine would be the use of a reagent that reacts with chlorine to form a colored product. However, this alternate approach is more likely to suffer from complications due to variation in reaction products as preferable to the production of color as a means for providing a visual "go-no-go" determination of disinfection.[12]

The initial procedure for preparing Nafion membranes containing methyl red consisted of the following steps. First, strips of Nafion membrane were cut from a sheet of film supplied by DuPont to a size suitable for insertion into a 1 cm spectrophotometric cell. if necessary, these strips were bleached in a chlorine solution, as recommended by DuPont, to remove any color, and then soaked in distilled water to rinse out the chlorine. The strips were dyed by soaking in a solution of methyl red in acetone until a desired absorbance was reached. Finally, the dyed strips were soaked in distilled water to rinse out the acetone.

During the course of our research, various improvements in techniques and modifications of procedure were introduced into the preparation of the AMDI system. Changes developed in response to the needs for better control over the characteristics of the membranes and indicator will be discussed in more detail later in this report. Our development of the AMDI system was directed toward the preparation of a controlled system suitable for the evaluation of its ability to serve as an integrating indicator of the disinfection efficiency of chlorine.

2. Controlling Water Absorption

Since the transport properties of Nafion membranes are largely determined by the amount of water absorbed,[8,9] this

characteristic provides the basis for altering the sensitivity of the AMDI to bleaching by chlorine. Increasing water absorption can significantly improve response, but it is necessary that the amount of water absorbed not vary during use in order to avoid interference with the bleaching kinetics. Other characteristics of the system that will be examined later also depend upon the water absorption.

The dimensional expansion that accompanies the absorption of water is an important consideration as well. The amount of water absorbed by a Nafion membrane can vary greatly, depending upon the equivalent weight, ion form, history of pretreatment, and the composition of the solution with which the membrane is in contact.[8,9] Water absorption tends to increase with a decrease in the equivalent weight. The Nafion 110 and 114 membranes, which have been used in our research, possess equivalent weights of 1100, the lowest commercially available. Considerations of ion form, pretreatment, and solution composition in the preparation of the membranes are covered in the following discussion.

The amount of water absorbed by Nafion membranes when soaked in water is highest with the polymer in the hydrogen form. The capacity to absorb water varies for different ion exchange forms when various cations serve as the counter-ions within the polymer.[8,9] Early in our work, we prepared the membranes in the hydrogen form in order to increase the sensitivity of the system. A Nafion membrane was converted into the hydrogen form by soaking in 15% HNO_3, as recommended by DuPont. However, problems were encountered with the use of Nafion in this ionic form. In the strong (sulfonic) acid form, the membranes tended to lower the pH of solutions into which they were placed. More importantly, however, the water absorption of the membrane tended to change during use, owing to the exchange of hydrogen for other cations,

usually sodium, in solutions with which the membrane came in contact. The water absorbed by Nafion 110 was found to slowly decrease from 18% for the hydrogen form to 16% after that form had been soaked in pH 5 sodium acetate buffer. It is important that such changes be avoided because of the effects on color intensity and transport properties of the AMDI. For this reason, membranes have been prepared more recently in the sodium form by soaking in 0.1 N NaCl solutions. Also, no cations other than sodium have been present in significant amounts in the solutions with which the membranes have come in contact.

Water absorption is also dependent upon the temperature of the water to which the membrane is initially exposed. If a sample is pretreated in water at an elevated temperature, it will retain an increased capacity to absorb water at room temperature, unless it is allowed to become dry.[8,13] This technique was also employed in our early work in order to increase sensitivity. Nafion in the hydrogen form was boiled in water for 30 minutes according to the standard pretreatment recommended by DuPont. Water absorption of 37% resulted for Nafion 110. This approach, however, suffered from the drawback of having to keep the membranes wet in order to maintain constant water absorption.[13]

Greater increases in absorptive capacity, partially attainable after drying, could be produced by swelling Nafion membranes in ethylene glycol at a temperature of at least 110° C, according to a pretreatment procedure developed by Coalson and Grot.[13] This technique was tested with the thinner Nafion 114 by soaking strips in boiling ethylene glycol for 30 minutes. Water absorption of 350% resulted, but the dimensional expansion of the membrane made it too fragile and deformed for use, and some of the polymer apparently dissolved during the treatment. Treatment of Nafion 110 was also initially tested in boiling ethylene glycol, with similar problems; the membranes partially dissolved

and became deformed. More satisfactory results were obtained by soaking Nafion 110 in ethylene glycol that was initially at 160° C, and that was then allowed to cool gradually to below 60° C before removing the membrane. This treatment allowed 160% water to be absorbed; about 45% water absorption was measured after the membrane was dried at ambient temperature and then resoaked in water. A larger sheet of Nafion 110 was treated in a similar manner by soaking in ethylene glycol that was initially at 145° C. After being rinsed and converted to the sodium form, this membrane absorbed about 65% water. The effect of the swelling was found to be uniform throughout the sheet. The absorption after drying and resoaking was approximately 40%, and this capacity was found to be reproducibly retained after repeated drying and resoaking. Since it is preferable that the water absorptive capacity for water not be further affected by drying after membrane preparation, most of the swollen Nafion 110 used in subsequent testing has been allowed to dry after treatment in ethylene glycol, even though this sacrifices some of the improvement in sensitivity provided by high water absorption. After the ethylene glycol treatments, the glycol is washed out of the film and no significant glycol remains.

3. Dyeing with Methyl Red

As mentioned previously, dyeing was initially conducted by immersing strips of membrane into a solution of methyl red in acetone. Tests were run to find an appropriate solution concentration that would require about 30 minutes in order to dye the Nafion membranes to an absorbance of about 1, as measured at the wavelength of maximum absorption (λ_{max}). Concentrations in the range of 0.5 mg/L or ppm were found to be satisfactory. While absorbances of about 1 were generally sought, the absorbances actually obtained often varied considerably during the course of our work with films that were later used in various tests of the system.

Examination of cross-sectional slices of dyed membranes under 10 X magnification with a light microscope showed the dye to be distributed throughout the membrane rather than just at the surfaces. However, from early on it was observed that the strips of film were not dyed evenly, since the edges were visibly darker, and the spectrophotometric measurement for a strip varied somewhat, depending upon how it was placed in the cell.

With the exception of a few early tests, the dyeing solutions were continuously stirred at a slow rate with a magnetic stirrer. The use of solution volumes of about 100mL for dyeing resulted in noticeable depletion of the dye concentration in the acetone solution during the process, indicating the need for considerably larger dye baths in order to effectively control the concentration for rate studies. Also, gradual color change in the solutions during dyeing indicated the need for buffering if the pH of the dye bath was to be controlled.

The use of acetone as the solvent for dyeing was eventually discontinued since its use affected the water content of the membranes and therefore interfered with the control of that characteristic of the system. Aqueous dyeing solutions were prepared with the addition of a small volume of concentrated methyl red in ethanol. In tests of dyeing of Nafion 114 strips in aqueous methyl red solutions it was found that a concentration of about 1 mg/L was required in order for 30 minutes of dyeing to result in an absorbance (at λ_{max}) of approximately 1.

4. Selection of neutral red

Films dyed with methyl red had a problem of desorption in basic solutions (which is discussed later in this report). For this reason an evaluation of possible alternate indicators was conducted. The following criteria are considered to be important

in the selection of a dye for use in an AMDI system with Nafion membranes: (1) It should be a cationic dye in order to be strongly sorbed within the anionic membrane. (2) It should be irreversibly bleached by free chlorine, but not be readily affected by chloramines or other possible interferences. (3) It should undergo a distinct color change when oxidized by chlorine to allow for visual detection. (4) It should have a high pK_a in water (at least greater than seven in water) so as to be insensitive to pH changes. (5) It should be readily obtainable in relatively pure form. (6) It should have low solubility in water in order to avoid desorption.

Available literature on dyes was reviewed, and neutral red was found to fit the above criteria reasonably well.[14-20] In particular, its pK_a in water is reported to be about 6.8, which is preferable to the pK_a of 4.8 for methyl red, and it is neutral in its basic form, while methyl red is an anion in basic solution.[19]

5. Dyeing with Neutral Red

Dyeing with neutral red was performed in aqueous solutions that were prepared by the addition of a small volume of concentrated neutral red in ethanol. The dyeing solutions were buffered at pH 4 or 5 with an acetate buffer in order to hold the pH constant with neutral red in cationic form. In all cases the solutions were continuously stirred with a magnetic stirrer during dyeing.

The actual dye content of the purest neutral red powder commercially available that was used in early testing was not specified by the manufacturer, but was later determined to be 69%. Subsequently, we obtained neutral red that had been certified by the Biological Stain Commission to contain 66% dye

content; this powder was then recrystallized from ethanol and the purified material found to have 90% dye content (refer to the experimental section of this report for further information). This recrystallization was conducted in order to remove impurities, such as excesses or contaminants of the reactants used in the dye synthesis; these might be sorbed within the dyed membranes and interfere with the measurement of bleaching kinetics. Knowledge of the actual dye purity permitted more accurate determination of the concentration of neutral red solutions used in subsequent dyeing investigations.

Tests of dyeing were initially conducted on the neutral red with 69% dye content. Longer dyeing times were introduced in order to allow for more uniform penetration of dye into the membrane. Also, the films were dyed to higher absorbances so that greater changes in absorbance could be observed in bleaching experiments. Strips of Nafion 114, 110, and 110 swollen by the above ethylene glycol treatment were all dyed with approximately 0.005 mM solutions of neutral red within 100 minutes, to absorbances (at λ_{max}) around 1.5.

Later, dyeing of strips of the three types of Nafion was conducted in solutions that were prepared from the recrystallized neutral red with 90% dye content. It was determined that absorbances of about 1.2 were reached within 100 minutes in 0.003 mM neutral red. Although, as presented above, the three types of Nafion were dyed to roughly the same absorbance levels within particular times, the dyeing rate (with regard to absorbance increase with time) for the ethylene glycol-swollen Nafion 110 actually appeared to be somewhat faster than the rates observed for Nafion-100 and -114 membranes that were not swollen in ethylene glycol. All three rates decreased with time in a similar manner apparently due to gradual reduction in the dyebath concentration. It was found that the dyed films became violet

when allowed to dry, but immediately returned to red when resoaked in water.

In order to avoid the darker edges resulting from the dyeing of strips, it was decided to dye a sheet of membrane, which would then be dried and cut into suitable strips for use. This approach was tested in the preparation of Nafion 114, with a sheet of the membrane being dyed in 0.005 mM neutral red for three hours. The sheet became dyed very unevenly, probably due to differences in solution flow across the surface of the membrane. In future work it may be preferable to dye in an unstirred solution. The average absorbance of the sheet was roughly estimated to be within the range of 1 to 1.5.

6. Pretreatment with Chlorine

In later preparations of Nafion 114, 110 and swollen Nafion 110, the membranes were soaked in solutions containing approximately 10 mg/L of free chlorine for several days prior to dyeing. This pretreatment was performed in order to eliminate any chlorine demand that could affect measurements of bleaching kinetics, and also to remove from the membranes any color that could interfere with spectrophotometric measurements. Some demand was observed for each of the three types of Nafion, as indicated by measured decreases in the concentrations of chlorine during soaking. This demand was probably mainly due to impurities that had been reported present within such membranes.[22]

Results from our final bleaching experiments (in which Nafion 114 dyed with neutral red was tested) have suggested that after having been dyed, the membranes again need to be pretreated in chlorine prior to use. In these experiments, it was found that some characteristics of the dyed membranes, particularly the

wavelength of maximum absorbance, were altered after exposure to chlorine. The specific results will be presented and discussed later in relation to the effects that were observed (see Spectral Characteristics and Dye Desorption). In order to avoid these changes in properties from occurring during use and interfering with the bleaching kinetics, some preliminary exposure to chlorine appears to be necessary.

7. Summary of AMDI Preparation

The preparation of the AMDI with Nafion membranes involves several principal considerations as outlined below.

(1) Selection of type of Nafion membrane. Nafion 110 (1100 equivalent weight, 10 mils thickness) and Nafion 114 (1100 equivalent weight, 4 mils thickness) have been used thus far in our research.

(2) Selection of indicator. Methyl red was initially selected, but neutral red employed as the indicator in more recent work. Various problems that will be described later, especially desorption in basic solutions, were evaluated in the selection of the indicator.

(3) Fixing the ion form. The polymer should be converted into the sodium form in order to stabilize water absorption.

(4) Fixing the water absorption. The amount of water absorbed, and therefore the sensitivity of the AMDI, can be significantly altered by the treatment of the membrane. Swelling in hot ethylene glycol has been found to be a useful procedure for improving

sensitivity. The preparation of the AMDI should allow for the membrane to maintain a constant water content during subsequent use.

(5) Pretreatment with chlorine. Membranes should be soaked in a chlorine solution before dyeing to remove any demand and color. After dyeing, some preliminary bleaching may also be desirable.

(6) Dyeing. This should be conducted under conditions appropriate to production of the desired color intensity (i.e. absorbance) while resulting in uniform distribution of the indicator throughout the membrane.

(7) Cutting into strips. The membrane, after dyeing, was cut into strips of suitable size in order to fit into a spectrophotometric cell for measurements of absorbance (for our purposes the dimensions are 9 mm x 38 mm). A labelled nylon line was attached to each strip to facilitate handling and identification.

B. Characterization of the AMDI

1. Water Absorption

The water absorption is defined by the following relationship:

$$\% \text{ WATER ABSORPTION} = 100 \frac{\text{WEIGHT OF WET MEMBRANE} - \text{WEIGHT OF DRY MEMBRANE}}{\text{WEIGHT OF DRY MEMBRANE}} \quad (3)$$

Refer to the experimental section of this report for a description of the procedure used in measuring water absorption.

Note that some values discussed below are relative to dryness at room temperature and humidity rather than to absolute dryness after drying at 105°C and then desiccating. At 50% relative humidity, the Nafion membranes in use (as received from DuPont) should have approximately 9% water absorption in the hydrogen form and approximately 4.5% water absorption in the sodium form.[7]

The dimensional expansion which accompanies the absorption of water needs to be taken into account in determining the appropriate size for cutting strips. Increases in linear dimensions are essentially isotropic and are related approximately to the increase in weight according to the following equation:[7]

$$\left(1 + \frac{\text{DIMENSION WET} - \text{DIMENSION DRY}}{\text{DIMENSION DRY}}\right)^3 = 1 + 2\left(\frac{\text{WEIGHT WET} - \text{WEIGHT DRY}}{\text{WEIGHT DRY}}\right) \qquad (4)$$

Therefore, measurements of dimensional expansion can also be used to estimate the water absorption.

Presented below are the water absorptions and dimensional change that resulted from various treatments employed in the preparation of Nafion 110 and 114 membranes. Additional information on the water absorption characteristics of Nafion products is available in the literature cited.[8,9]

A treatment employed early in our work involved converting the membrane into the hydrogen form and then boiling in water for 30 minutes. For nine strips of Nafion 110 that were treated together in this manner, the mean water absorption was found to be 36.7%, with a standard deviation of 0.3%. This amount of

water absorbed is consistent with data reported by Grot, et al.[8], which indicates that the water absorption after boiling 30 minutes in the hydrogen form should be about 35% for an equivalent weight of 1100. The same strips were also found to retain 6.5% water absorption after drying at room temperature and humidity.[8] This is somewhat less than the level of 9% that has been reported at 50% relative humidity. The increase in weight relative to weight achievable by drying at ambient conditions was therefore 28%. The width and length of the strips increased from 8 mm x 35 mm when dry at room conditions to 9 mm x 41 mm when wet. According to the relationship presented above, this roughly 17% dimensional expansion indicates an increase in weight of about 30%, which agrees with the value of 28% noted previously, within the limits of the experimental measurements.

More recently, Nafion 110 has been pretreated by swelling in hot ethylene glycol, washing away the glycol with water and then converting into the sodium form. In tests of swelling in boiling ethylene glycol, water absorption ranging from about 200% to 300% was obtained. After room temperature air-drying and then resoaking in water, the amounts of water absorbed were found to have decreased to about 60-70%. Treatment of Nafion 110 in ethylene glycol that was initially at 160°C and that was then allowed to gradually cool to below 60°C before removal of the membrane, resulted in approximately 160% water absorption. This decreased to about 45% after room temperature air-drying and then resoaking.

Nafion 110 swollen in ethylene glycol that was initially at 145°C and that was then allowed to gradually cool to below 60°C before removal of the membrane, attained approximately 65% water absorption after rinsing away the glycol. Length and width were found to have increased about 30% relative to the dimensions after air drying at room temperature. This dimensional expansion

is consistent with the measured increase in weight in accordance with the equation above. After room temperature air-drying and then resoaking, the water absorption was determined to be 34% relative to drying at room temperature and humidity. Assuming about 4% water absorbed at room conditions, the total water absorption is approximately 40%. The dimensions of strips of this treated membrane increased from 8 mm x 35 mm x 0.29 mm when dry at room conditions to 10 mm x 42 mm x 0.39 mm when wet. These dimensional changes do not appear to have been isotropic (the relative increase in thickness is the greatest), which could be accounted for by structural modification of the polymer network caused by the ethylene glycol treatment.[13] Also, comparison of the dimensions of the original dry Nafion 110 to the dimensions after the treatment in ethylene glycol and then air-drying at room temperature, indicates an increase (from 0.25 mm to 0.29 mm) in the thickness, in contrast to small decreases in length and width.

The water absorptive capacity of about 40% was found to be reproducibly retained after repeated air-drying at room temperatures and resoaking. The membrane absorbed water quite rapidly, with the water absorption exceeding 30% within 10 minutes after immersion of the dry membrane in water. A high diffusion coefficient for water in Nafion membranes has been previously reported.[23] Thus, while it appears that the AMDI could be stored dry, it apparently should be equilibrated in water for some time prior to use in order to allow the membrane to reach complete water absorption. Alternatively, the membrane could be stored in water or shipped in 100% humidity containers.

Measurements have also been conducted for Nafion 110 and 114 membranes in the sodium form that had not been previously subjected to any swelling pretreatment. The water absorption for Nafion 110 has been measured at 12% relative to weight achievable

by drying at room temperature and humidity. The corresponding dimensional expansion was found to be about 7%. Assuming that 4% absorption is retained at room conditions, the total water absorption for Nafion 110 in the sodium form appears to be approximately 16-17%. This value is consistent with the reported water absorption properties of Nafion membrane.[8] For Nafion 125 with a measured equivalent weight of about 1150 and in the sodium form, Lopez et al.[24] determined the water concentration by IR spectroscopy to be 11 moles of water per mole of exchange sites, which converts to 17% water absorption on a weight basis. The Nafion 114 membrane should exhibit nearly the same water absorption as the Nafion 110 since both have an equivalent weight of about 1100. Dimensional changes for Nafion 114 have been observed to be approximately the same as for Nafion 110.

Dyeing could possibly alter the water absorption of the membranes somewhat, with the extent of the effect probably dependent upon various factors, including membrane characteristics, dyeing conditions, amount of dye sorption, and whether other treatments affecting the water absorption were conducted either before or after dyeing. The sorption of methyl red by Nafion 110 that had first been boiled in the hydrogen form did not significantly affect the amount of water absorbed until the membrane had been dyed far beyond an absorbance of 2; thus the effect of dyeing appeared to be negligible in this case. The water absorption for strips of Nafion 114 that had been converted into the hydrogen form, dyed in aqueous methyl red solution, and then boiled in water for 60 minutes, was measured to be 27%, which is somewhat less than expected for boiling in the hydrogen form. Other observations have not indicated any large changes in water absorption as a result of dyeing. However, the effect of dyeing needs to be evaluated more thoroughly by measuring the amounts of water absorbed in the various dyed membranes that have been prepared.

2. Properties of Neutral Red

The visible and ultraviolet absorption spectra that were recorded for solutions of neutral red were comparable to spectra found in the literature.[25,26] The visible wavelength of maximum absorption (λ_{max}) in water was determined to be 534 nm for NR^+, the cationic form (pH 2-5), and 452 nm for NR^- base, the basic form (pH 9). Similar values, namely 532 nm and 450 nm, were reported by Bartels.[25] In 50% ethanol, λ_{max} for NR^+ was 542 nm, which agrees with other reported values.[27] The molar absorptivities (ε) in water were measured in 4-cm cells with 0.0062 mM solutions of the neutral red that had been purified and assayed. The molar absorptivity for NR^+ at 534 nm was determined to be about 34,000 L $mole^{-1}$ cm^{-1}. In addition, it was observed that the relationship of absorbance to concentration was linear over the concentration range of 0.0006-0.03 mM, thus conforming with Beer's Law. The molar absorptivity for NR^- base at 452 nm was determined to be 16,000 L $mole^{-1}$ cm^{-1}. The values of 34,000 and 16,000 L $mole^{-1}$ cm^{-1} found here are somewhat higher than the respective aqueous values of about 28,000 and 13,000 L $mole^{-1}$ cm^{-1} indicated by Bartels.[25] The discrepancy may be accounted for by the characteristic accuracy of the measurements and the purity of the dye. Bartels apparently relied upon analysis of nitrogen content, while the assay of dye content in this work was performed by a standardized spectrophotometric method.[15] Lower values of 23,000 and 8,000 L $mole^{-1}$ cm^{-1} were reported by McDonald, et al.,[28] who did not discuss the purity of the neutral red used.

The pK_a of the dye in water was determined by a spectrophotometric method[29] using 0.0062 mM buffered (I = 0.01) solutions of the purified neutral red. Absorbances of the solutions were measured at 534 nm in 4-cm cells. It was found that the absorbance at low pH (dye completely in NR^+ form) was

0.86, while the absorbance at high pH (dye completely in NR^- base form) was 0.10. Solutions at intermediate pH values of 6.8 and 7.2 had absorbances of 0.47 and 0.33, respectively; in each of these two cases the data lead to the calculation of a pK_a value of 6.8. This result is consistent with the pK_a values of 6.6-6.8 found in the literature.[19,27,30,31] Somewhat different values have been reported by others, including 7.4 by Guss and Kolthoff.[32] However, Bartels[25] reported that the dissociation also involves the formation of another form of NR^+, which he has suggested to be a carbonium ion (see Figure 3). He determined a pK_a of 5.9 for equilibrium of two NR^+ forms, and a pK_a of 7.4 for dissociation of the carbonium form to NR^- base. The overlapping of the transformations in the pH range of 5.9-7.4 would account for the apparent pK_a at pH 6.7.[32]

The oxidation of neutral red with monochloramine was found to be much slower than with free chlorine. Upon the addition of 2 mg/L (ppm) of free chlorine, the red color immediately disappeared from a 2 mg/L (ppm) neutral red solution. In contrast, a gradual color change from red to yellow occurred over a period of about 15 minutes after the addition of 1 mg/L (ppm) of monochloramine to a 2 mg/L (ppm) neutral red solution. The use of neutral red as an indicator for measuring free chlorine without interference from chloramines was previously reported by Elliot.[20]

3. Dye Sorption

The amount of dye sorption (i.e., concentration of dye in membrane) corresponding to particular absorbances (i.e., optical densities) has been determined for the different preparations of membranes listed in Table 1 below. Measurements of dye uptake were conducted according to the procedure described in the experimental section of this report.

Table 1. Dye Sorption

Dye	Nafion type	Water absorption %	Absorption at max %	Dye sorption mg dye/g dry polymer
Methyl red	114	20	2	0.8
Neutral red	114	16	1.5	1.0
Neutral red	110	16	1.6	0.4
Neutral red	110*	40	1.6	0.5

* swollen in hot ethylene glycol

These results provide a basis for estimating the minimum concentration and volume of dye solution needed for dyeing a particular amount of membrane. Knowledge of the quantity of dye sorbed is also useful in theoretical evaluations of the system. For example, the dye sorption reported for neutral red in Nafion 110 represents only about 0.2% of the ion exchange sites of the membrane as being occupied by dye molecules; also, for the same neutral red-Nafion 110 membrane, the complete bleaching of one strip (0.14 gram) should reduce the concentration of 100 mL of a 1 mg/L Cl_2 (1 ppm) solution by about 7%, assuming that one equivalent of neutral red is oxidized by one equivalent of chlorine.

In apparent accordance with the Lambert-Beer law (absorbance = absorptivity x thickness x concentration), the results show that the thinner Nafion 114 (4 mils thick) sorbs about 2.5 times as much neutral red as Nafion 110 (10 mils thick) to reach approximately the same level of absorbance. When comparing other results for Nafion 110 and 114 that are expressed in terms of absorbances one should bear this relationship in mind. The higher sorption value for neutral red in Nafion 114 relative to methyl red in Nafion 114 could be accounted for by the lower molar absorptivity reported for methyl red in solution. In order

to determine absorptivities for the sorbed dyes, better measurements of the volumes and thicknesses of wet dyed membranes would be needed. However, rough calculations using present data have indicated that the absorptivities of the sorbed dyes are somewhat less than the absorptivities of the dyes in aqueous solution. This would be consistent with the findings of Baumgartner, et al.,[31] who reported that the absorptivity of neutral red was decreased by interaction with poly(sodium styrenesulfonate).

It was determined that Nafion 110 (boiled in hydrogen form) had sorbed approximately 90 mg of methyl red per gram of dry polymer, after successive soaking in ethanolic 0.4 mM methyl red solutions until no further sorption occurred. This corresponds to over one molecule of dye per three ion exchange sites. It is expected that dye absorption could be described by a Langmuir isotherm with saturation theoretically coinciding with the ion exchange capacity. However, the reported clustering of the ion exchange sites in Nafion membranes could interfere.[23]

4. Spectral Characteristics of Dyed Membrane

Spectral measurements of the undyed Nafion membranes have shown them to be highly transparent in the visible region, with only a small continuous absorbance of about 0.01 to 0.02 throughout the wavelength range of 400-600 nm. Within the wavelength range of particular interest, from 520 to 540 nm, absorbances of the membranes have been found to average 0.02, as indicated by the data in Table 2, which were obtained for three different representative strips from each of the four membrane preparations listed.

Table 2. Absorbances of Undyed Nafion Membranes

Nafion type	Water absorption (approx. %)	Absorbances
114	20	0.02, 0.02, 0.02
114	16	0.01, 0.02, 0.02
110	16	0.01, 0.02, 0.02
110*	60	0.01, 0.02, 0.02

* swollen in hot ethylene glycol

Consequently, the absorbances reported for dyed membranes have in most cases been corrected by subtraction of 0.02 from the spectrophotometer readings.

A visible spectrum of membrane dyed with neutral red is shown in Figure 1. The shapes of the spectral curves of the sorbed dyes appear similar to those of the dyes in solution. Visible wavelengths of maximum absorption (λ_{max}) that have been determined for various dyed membranes are listed in Table 3.

Table 3. Wavelength of Maximum Absorption λ_{max} for Dyed Membrane. pH of External Solution Approximately 5.

Dye	Nafion type	Water absorption (approx. %)	λ_{max} nm
Methyl red	110	25	520
Neutral red	114	16	528
Neutral red	110	16	529
Neutral red	110*	40	531

* swollen in hot ethylene glycol

For each of the three types of membranes dyed with neutral red, the maximum absorption was found to occur at shorter wavelengths than the 534 nm maximum measured for neutral red in aqueous solution. This condition resembles the phenomenon known as metachromasy (or metachromasia), which is the spectral shift

exhibited by certain dyes when sorbed by so-called chromotropic materials of opposite charge, (e.g. in biological staining), or when polyelectrolytes of opposite charge are added into a solution of the dye.[15,33] The metachromatic effect is generally hypsochromic (i.e., toward lower wavelength) for cationic dyes. Baumgartner et al.[31] reported such downward shifts of λ_{max} for neutral red in the presence of poly(sodium styrenesulfonate), which were attributed to the "polyelectrolyte effect." The smaller shift observed here for the Nafion 110 swollen in hot ethylene glycol, relative to the other Nafion 110 and 114 membranes, may be due to the significantly higher water content of the swollen membranes.

It has been observed that membranes dyed with neutral red also undergo additional shifts of λ_{max} during bleaching with chlorine. The maximum absorption of the dye membranes was found to have shifted to longer wavelengths following exposure to chlorine, as the data in Table 4 indicate.

Table 4. Spectral Shifts During Bleaching. Membranes Dyed with Neutral Red.

Nafion type	Water absorp. approx. %	Exposure(s) to chlorine, ppm-min.	λ_{max} after exposure(s), nm
110	16	111	531
110	40	111	534
114	16	111	531
114	16	67	530
114	16	29,73	529,531
114	16	67,73	529,532
114	16	34**,29,73	531**,532,536
114	16	122**,67	539**,541

* *swollen in hot* ethylene glycol

**bleaching occurred on the same day that the membrane had been dyed and allowed to dry, possibly accounting for the larger shifts.

This spectral shift during bleaching introduces some error into measurements of bleaching kinetics experiments run before this phenomenon was known. All experiments have been based upon absorbance readings at the same wavelength throughout the experiment. It is not yet known whether methyl red undergoes similar changes. Further investigation is needed to determine how best to handle this situation, which apparently also involves an associated effect upon the desorption of dye considered later in this report.

5. Dissociation of Sorbed Dye with pH

Both methyl red and neutral red are acid-base indicators that undergo dissociation and reversible color changes within particular pH ranges in solution. The relevant equilibria and pK_a values in water are shown in Figures 2 and 3. As the results presented below demonstrate, the sorbed dyes (i.e., in Nafion membranes) are also susceptible to similar dissociation and color change, but at higher values of the external solution pH (i.e., there is an apparent upward shift of the pK_a). This dissociation causes problems since the color change (and also the related dye desorption to be discussed later) can interfere with the use of the AMDI to measure chlorine bleaching kinetics. However, the shift to higher pK_a for neutral red is advantageous because it helps to raise the pH interval within which the dissociation occurs. It is desired that the AMDI be free of such interferences at least within the pH range of 4-9. It was primarily because of the shortcomings of methyl red in this regard that neutral red was selected for evaluation as an alternative dye.

The dissociation of the sorbed dye has been investigated by observing changes in absorbance at λ_{max} when dyed membranes were equilibrated in solutions of different pH. When the dyed

membranes are in solutions of pH 5, it appears that methyl red is almost completely in its zwitterion form, while neutral red is almost completely in its cationic form. Thus, absorbances in solution at pH 5 have been used to provide a reference for judging whether dissociation is occurring in a solution at higher pH. The problem of desorption (to be discussed later) presents a potential interference, which has been taken into account in the experimental procedure and analysis of the data. Similar methods for determining dye pK_a have been used by others.[29] In our work, thorough determinations of the pK_a values for different dyed membranes have not been conducted; instead, tests have simply been performed to approximately evaluate the pH range in which dissociation occurs. Table 5 contains the data obtained from tests of various dyed membranes. Where possible, the apparent pK_a has been roughly estimated in order to serve as a basis for comparison of the results.

These results indicate that the dissociation for both sorbed dyes occurs at pH values about three or four units higher than for the dyes dissolved in water (pK_a in water for methyl red is 4.8 and for neutral red is 6.7). Accordingly, the membranes dyed with neutral red appear to undergo dissociation at higher pH than similar membranes dyed with methyl red. Since significant interference with the AMDI performance could result from dissociation at pH values within about two units of the apparent pK_a, only the Nafion 110 dyed with neutral red shows the potential for being relatively free of this interference in the pH range of 4-9 as desired. The color changes of the dyed membranes due to dissociation are reversible just as for the dyes in solution (except for losses from dye desorption), but at least several minutes at equilibrium are required for the color to adjust to the change of the external solution pH. It is expected that the ion form and water adsorption of the membrane could influence the dissociation of sorbed dye, but the effect of these

factors has not been measured. The membrane thickness may also be important, since the results indicate the possibility of higher apparent pK_a values for Nafion 110 than for the thinner Nafion 114.

Similar shifts of the pK_a of neutral red were found in solutions containing poly(sodium styrenesulfonate) by Baumgartner et al.,[31] who have also discussed the rationale for these shifts and the previously-mentioned shifts in the absorptivity and wavelength of maximum absorption. The observed changes in the apparent pK_a have been attributed by Baumgartner et al.[31] to the electrostatic interaction of the polyelectrolyte and the oppositely charged dye molecule.

Table 5. Dissociation of Sorbed Dye.
Absorbances of Dyed Membranes Measured Near λ_{max}.

Dye	Nafion Type	Ion form	% Water Absorp. approx.	Absorbance in external soln. at pH 5	Absorbance of:	Solution at pH of:	Estimate of apparent pK_a
Methyl red	110	H + Na	25	0.39	0.34	6.7	
				0.37	0.30	8.4	8
				0.32	0.01	11.6	
Methyl red	114	H + Na	25	0.36	0.17	8.4	8
Neutral red	114	Na	20	0.24	0.24	8.4	8
Neutral red	114	Na	16	0.28	0.07	10.0	9 or 10
Neutral red	110*	Na	200	0.83	0.83	8.4	
Neutral red	110	Na	16	0.51	0.44	10.0	10 or 11
				0.44	0.39	9.7	

*Swollen in hot ethylene glycol

Table 6. Methyl Red Desorption Rate

Nafion type	Water Absorption	Absorbances Before	Absorbances After	pH	Time h	Absorbance loss	Half-life hours
114	25	0.49	0.36	8.4	0.50	0.13	1.1
		0.36	0.30	8.4	0.50	0.06	1.9
		0.29	0.26	8.4	0.50	0.03	3.2
		0.75	0.67	8.4	0.50	0.08	3.1
		0.67	0.60	8.4	0.50	0.07	3.1
		1.2	0.80	12.0	0.25	0.40	0.43
110	25	0.44	0.39	6.7	25	0.05	144
		0.39	0.37	8.4	0.50	0.02	6.6
		0.37	0.32	11.6	0.33	0.05	1.6
		0.40	0.20	8.4	24	0.20	24

6. Dye Desorption with pH in Time

Inasmuch as desorption of dye represents a loss of membrane color in a manner similar to bleaching, any desorption produces an interference in the determination of chlorine's bleaching rate. Table 6 shows the rate of dye desorption from methyl red dyed films, both Nafion 110 (1 mil thick) and Nafion 114 (4 mils thick). Where the membrane material was initially in the hydrogen form, it contained 25% water. Measurements were made in buffered solutions containing large concentrations of sodium, and therefore, in interpreting these results, one must consider that the ionic form of the membrane material has shifted. All absorbances were measured at pH 5, at which pH it was determined that an immeasurably small loss in absorbance occurred over a 24 h period. The last column of the table was calculated on the assumption that the loss of absorbance obeyed first-order kinetics. The table also gives the half-lives for absorbance loss. Since the half-lives are not constant, even at constant pH's, for a given membrane type, going from 1.1 h to 3.2 h at pH 8.4, the results suggest that loss of absorbance is not a simple first-order process or that experimental error is relatively high

in these measurements. The latter would not be unreasonable, since the absorbance changes were relatively small for many of the measurements. The trends, however, are clear, with higher pH increasing significantly the rate of desorption of dye. At pH 12 the desorption half-life for the Nafion 114 film is less than half an hour, and that for the Nafion 110 film is 1.6 h. At pH 8.4 the desorption half-time for the Nafion 114 film is nearly 3 h, while the Nafion 110 film desorption rate varies from 6.6 to 24 h. In both cases, the desorption rates at neutral pH were quite slow. Given the estimated pK_a value of methyl red in the dye film of 8 to 9, this increased desorption rate at higher pH is logical. This limits the application of a methyl red-based film for an AMDI system to pH values less than 9.

Table 7 shows the contrasting neutral red desorption rate. Unlike methyl red, the neutral red dye remains as a cation in solution pH's up to 10, as shown by the pH dissociation data of the dyed membrane. Table 7 shows the neutral red desorption rate as a function of pH and also film thickness, and in two experiments, percent water absorption. In contrast to the experiments above with methyl red, these films were all in the sodium form. Therefore, no conversion occurred in this system between the hydrogen and sodium form because of the high sodium buffer concentration as had occurred in the methyl red studies. The percent water absorption (or permeability and film expansion) was lower, 16% rather than 25%. It can be seen that the neutral red desorption rate is considerably slower than the methyl red desorption rate at similar pH's. Again, half-life times were calculated on the assumption of first order desorption of dye in spite of the variability found with decreasing initial dye absorbance, which would suggest that the order of the desorption reaction is greater than first order. With neutral red, the desorption rate at pH 6.8 and 8.4 is quite small. At pH 10, the desorption rate for the Nafion 114 film is unacceptably high, but

the desorption rate for the 10 mil film is acceptable. Surprisingly, even the 10 mil film that had been swollen in hot ethylene glycol to contain 200% water still showed negligible absorbance loss after 45 minutes at pH 8.4.

Table 7. Neutral Red Desorption Rate

Nafion Type	Water Absorption %	Absorbance Before	Absorbance After	pH	Time h	Absorbance loss	Half-life Hours
114	16	0.73	0.72	6.8	0.66	0.01	33
	16	1.52	1.48	6.8	0.33	0.04	8.6
	16	1.27	1.25	6.8	0.33	0.02	14.4
	16	1.19	1.16	6.8	0.33	0.03	9.0
	20	0.26	0.24	8.9	0.5	0.02	4.3
	16	0.42	0.28	10.0	0.25	0.14	0.4
110	16	0.65	0.65	6.8	0.66	0.0	---
	40*	0.42	0.40	6.8	0.66	0.02	9.4
	200*	0.83	0.83	8.4	0.75	0.0	---
	16	0.47	0.44	9.7	0.25	0.03	2.6
	16	0.55	0.51	10.0	0.25	0.04	2.3

*Swollen in hot ethylene glycol

These results show that desorption of dye is primarily a function of pH and secondarily a function of membrane thickness. The lower acid dissociation constant of methyl red in dyed films compared to neutral red corresponded to a more acceptable (lower) rate of dye desorption for the latter dye and to decrease interference through desorptive dye loss in the bleaching measurements at high pH. It would appear that the neutral red desorption rates in Nafion 100 films are low enough to permit these films to be used at pH values as high as 10 with contact times of 15 to 30 minutes at such high pH. At high pH and longer contact times, a blank correction might be needed to account for the loss of dye or apparent bleaching in the absence of chlorine. At lower pH and/or shorter contact times, the problems is minimized. Water content and amount of dye within the membrane

undoubtedly also affect the rate of bleaching of dye in the basic solutions. However, these effects are small and have not been quantitated. Attempts were made to find methods of treating the dyed film in order to minimize the dye desorption rates. Attempts were made to cycle pH, boil films, and otherwise improve the bonding of the dye to the cation site, but in general the effects of such treatments were not found to be significant.

C. Use of the AMDI

1. Methyl Red Bleaching

Early bleaching experiments with methyl red were conducted to demonstrate the fundamental characteristics of the system. Bleaching of methyl red-dyed Nafion 114 (26% water content) was evaluated as a function of time in a solution with constant chlorine concentration. The experimental conditions for this early experiment were constant free chlorine concentration of 1.85 mg/L, solution pH fixed at 5 with an acetate buffer with an ionic strength of 0.02, constant temperature of 25^{o}C and constant stirring. The absorbance of the dyed film was measured in a Cary 14 spectrophotometer at 520 nm as described above. The initial absorbance, A, was 0.89. The absorbance was measured at 10 minute intervals and reached a value of 0.25 after 110 minutes.

A semilog plot of the data obtained shows (Figure 4) a good logarithmic dependence of bleaching kinetics with contact time, with a slope of -0.0051, intercept 0.013, and correlation coefficient, r^2 value, of 0.999.

These results suggest that bleaching of the dyed film is a first order reaction and thus, could be an appropriate model for disinfection kinetics. According to Chick's Law, the destruction of organisms by chlorine can be expressed by the logarithmic

equation $\log N/N_o = -\Lambda Ct$ where N/N_o is the survival fraction, C is the disinfectant concentration and Λ is a constant referred to as the specific lethality coefficient by Morris.[1] If the concentration as well as the specific lethality coefficient are constants, the product of these terms is simply the slope of the disinfection kinetic rate curve. Thus the rate of a disinfection process could be calibrated as corresponding to an extent of bleaching of a dyed membrane AMDI strip.

2. AMDI Modelling

Seldom is the concentration of chlorine constant during a disinfection process. This leads to one of the major problems in judging disinfection process efficiency based on a single residual measurement taken at the end of the process. The ability of the AMDI to measure the integral of changing concentration with time was tested to preliminary studies with the methyl red AMDI.

Bleaching of methyl red in Nafion 110 (37% water content) in a solution of decreasing chlorine concentration and in a solution with constant chlorine concentrations were both compared to the amperometric chlorine electrode continuous measurement of free chlorine at fixed pH and constant temperature,[3,4] and to the rate of inactivation of spores of B. subtilis. Table 8 shows the total dose as the concentration measured by the electrode multiplied by the time of exposure of the AMDI film. This is compared to the extent of bleaching of the AMDI film.

Table 8. Bleaching of AMDI Compared to Total Dose

Exposure to chlorine (ppm-min of HOCl)	Conditions	Decrease in Absorbance
40	decreasing conc. pH 7	0.09
40	constant conc. pH 5	0.11
72	constant conc. pH 5	0.19

The decrease in absorbance of the AMDI was corrected for the loss in absorbance observed at the given condition and time without chlorine. This blank averaged a loss in absorbance of 0.02.

3. Spore Inactivation and AMDI Modelling

Fourteen spore experiments were run in two groups separated by about two months. The first experiment was run without chlorine to serve as a blank. The next nine experiments were run at constant HOCl concentrations and the last four were run with changing HOCl concentrations.

A typical plot of $\log_{10} N/N_o$ versus D is given in Figure 5. These data show the spore inactivation run corresponding to the same run for the AMDI bleaching given in the table above. During this experiment, the concentration of HOCl initially was 3.3 ppm Cl_2 and decreased to about 1 ppm over a period of 21 minutes. A logarithmic rate of inactivation was observed, the slope of which was $-\Lambda_{10}$. In some experiments, where the $\log_{10} N/N_o$ went beyond -3 or -4, tailing occurred. This resulted when the spore concentration dropped below 200/mL, which was the lowest concentration that could be reliably measured.

A semilog plot of the number of spores surviving per mL versus time in minutes for such a changing concentration experiment was not in general linear and the slope of this plot bears no relationship to $-\Lambda_{10}$.

Table 9 contains the data from the fourteen experiments. The experiments are numbered chronologically. Average HOCl is reported in mg/L (ppm) Cl_2. D intercept is the value of the y intercept of the straight line portion of the plot of $\log_{10} N/N_o$ versus D. It represents the microbiological dose at which kill begins. The microbiological dose, D, in all cases, is given in

ppm min. D, 1 log kill, is the microbiological dose at which $\log_{10} N/N_0 = -1$ or 90% of the spores are dead. D, 3 log kill, is the microbiological dose at which $\log_{10} N/N_o = -3$ or 99.9% of the spores are dead. These two values are taken from the linear regression. NA was used where the data could not be calculated. For example, in some of the first experiments, samples were not spread over enough time and 3 logs of kill was not reached. The values with asterisks are average concentrations for the changing concentration experiments.

Lag phases are not uncommon and have been observed with other organisms and with other halogen disinfectants.[34,35,36] The process that occurs during the lag phase is not known. It may be due to chlorine reacting with the spore coat before it can get to the cell. If this is the case, the chlorine dose spent during this period does not accomplish any disinfection. Disinfection does not begin until after the demand of the spore coat is satisfied. Another possibility is that diffusion through the spore coat is a controlling parameter. In this case, the chlorine may not be reacting with the coat, but in the time for diffusion to occur there is not disinfection. In either case, the microbiological dose required to reach a specific point in the disinfection process was found by subtracting the lag phase dose from the total microbiological dose applied.

The data from the experiments have been broken down into two sets. This was done for three reasons. It appears that the spores' resistance to chlorine was reduced with time, and the two sets are separated by two months. The second set of data had a smaller deviation than the first set, probably due to improved technique with continued practice. The last seven experiments formed a complete data set with respect to D for 3 logs of kill. The only missing point (#13) was easily extrapolated because the last point of the D versus $\log_{10} N/N_o$ plot fell very close to 3 logs of kill.

A plot of average HOCl concentration versus Λ shows no correlation (R^2 = 0.01) for the constant concentration experiments. This finding supports the hypothesis that n = 1. If n differed substantially from 1, it would appear to change as the concentration changed. If n = 1, then D of 3 logs kill/D of 1 log kill should equal 3. Using the values in Table 8, it can be seen that the answer is 2.3. If the lag phase (D intercept) is subtracted from both values as discussed earlier, the answer becomes 3.1 (standard deviation 0.15). This value supports the proposition that n = 1 for these spores. Morris[1] and others have also justified the use of n = 1.

The last four experiments of the series were run using varying concentrations of chlorine. It can be seen in Table 8 that there is no discernible difference between the experiments run at constant HOCl concentrations and those with varying HOCl concentrations.

That the residual at any given time is not an indication of the level of disinfection can be seen in Figure 6. In this plot, the data of the last five experiments and #6 are used. The last four were changing concentration experiments. The other two were chosen because they were high and low constant concentrations and filled out the graph. The concentration of HOCl at 15 minutes into the experiments is plotted against the reduction in spores at that point. It is clear that there is no relationship between the two. In contrast to that, Figure 7 is a plot of the microbiological dose at 15 minutes versus the reduction in spores at that point for this same data. There is a clear relationship between the two parameters. The comparison of D with residual HOCl at 15 minutes is not entirely fair. The residual measurement is not meant to model disinfection. All of these points clearly demonstrate that the microbiological dose is a

true measure of disinfection and not other measures such as residual HOCl at a specified time.

If the concept of microbiological dose could be incorporated into water treatment, it would greatly improve our ability to provide safe drinking water without excess chlorination. Experiments could be performed to determine an adequate microbiological dose for the most resistant pathogen found in the water of a given region. With an added margin of safety, this dose could be applied to water supplies to deliver safe drinking waters.

The development of an AMDI strip and correlation of the bleaching occurring in such a strip, with a degree of inactivation, would make the use of this concept of integration of the total microbiological dose a practical method of disinfection control; this would be much more likely to correlate with the degree of inactivation obtained than the current methods of measuring residual at the end of a certain contact time.

Table 9. Inactivation of B. subtilis by Chlorine at pH 7 and 30°C.

Experimental Number	Avg. Conc. ppm HOCl as Cl_2	D intercept (ppm min)	D 1 log kill (ppm min)	D 3 log kill (ppm min)	Λ_{10} $(ppm\ min)^{-1}$
1	0.0	NA	NA	NA	NA
2	1.35	1.0	10	NA	.258
3	1.07	7.8	22	NA	.164
4	1.22	4.4	11	25	.327
5	1.36	4.0	15	37	.212
6	0.60	6.6	13	28	.318
7	1.79	1.7	15	NA	.180
8	2.73	5.2	11	23	.392
9	1.14	5.8	11	23	.407
10	1.67	4.4	12	26	.316
11	1.59*	5.7	12	25	.362
12	1.78*	1.2	9	25	.290
13	0.93*	3.7	11	(26)	.311
14	0.84*	6.0	12	25	.359
Mean all		4.4	12.6	26.3	.300
Std. dev. all		2.1	3.3	4.0	.077
Mean last 7		4.6	11.1	24.7	.348
Std. dev. last 7		1.7	1.0	1.3	.044

* Changing concentration caused by thiosulfate addition.

IV. Military Significance

Current methods of controlling military water supply disinfection are unsatisfactory because they do not consider the very important pH, temperature and changing concentration variables in the disinfection process. In current disinfection practice, residual concentration is measured only once, after the time considered necessary for disinfection. Inasmuch as concentration is not constant during the disinfection process, and temperature and pH are extremely important in determining the effectiveness of a chemical disinfectant, very large errors are made in disinfection efficiency interpretation. This had made it

necessary for the Army to use excessively high residual values of five and ten mg of free available chlorine per liter. These high residuals create taste and odor problems that encourage personnel to use unsafe alternative water sources.

Because the military must often use low quality water as a raw water supply, the possibility exists for this water to contain microbiological as well as chemical contamination and demand. This produces considerable variation in the concentration of disinfectant available during the contact period. It is necessary that disinfection be better controlled and maintained at a high level of efficiency under these conditions. By use of an AMDI strip or indicator system as described, it should be possible to obtain this better control of disinfection efficiency through overall integration of concentration x time, which also includes pH and temperature effects.

V. Conclusions and Recommendations

This research has provided a demonstration of the principles of an analog membrane disinfection indicator, and has shown the feasibility of the concept by developing two dye systems. The methyl red dyed Nafion film and the neutral red dyed Nafion film indicator systems were shown to be capable of measuring the diffusion of chlorine into an indicator strip and integrating the response of the chlorine concentration as a function of time. A few limited comparisons against the spore of B. subtilis gave a rate of disinfection that appeared to follow the degree of bleaching the methyl red-dyed Nafion film.

Several significant problems were encountered in producing an AMDI system. Two problems that appear to be solved are loss of dye at high pH by loss of the dye from the film over time, and

controlling membrane characteristics so as to provide a stable response but at the same time give reasonable sensitivity.

The neutral red, Nafion AMDI system appears to provide a stable film with reasonable sensitivity to HOCl. A great deal of work remains, however, to prove the AMDI concept and to demonstrate its utility under conditions of varying water types both chemically and microbiologically.

It is recommended that the AMDI system be pursued as a method for evaluation of disinfection effectiveness far superior to the current methods of residual measurement. The colorimetric system of neutral red-dyed films should be further developed and characterized in terms of its bleaching rate by free chlorine as a function of pH, ammonia, ionic strength, temperature and concentration. These results then should be compared and correlated with microbial disinfection kinetics as a function of the varying conditions likely to be encountered in practical disinfection processes and with current methods of judging disinfection efficiency by chemical means.

Although the neutral red system appears to be promising, its sensitivity could be improved profitably. It is therefore recommended that efforts also be made to develop new AMDI systems. Such systems may simply be improvements on the dyed film system with new membrane materials or different physical characteristics with the same film, such as making it microporous, as has been done with Teflon films.

Finally, to aid in the visual determination of a color end point, color masking agents might be incorporated to change the simple bleaching of color into a change to a contrasting color using the principle of color masking to produce a "go-no-go" indication of adequate microbiological dose as defined above.

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VII. Figures

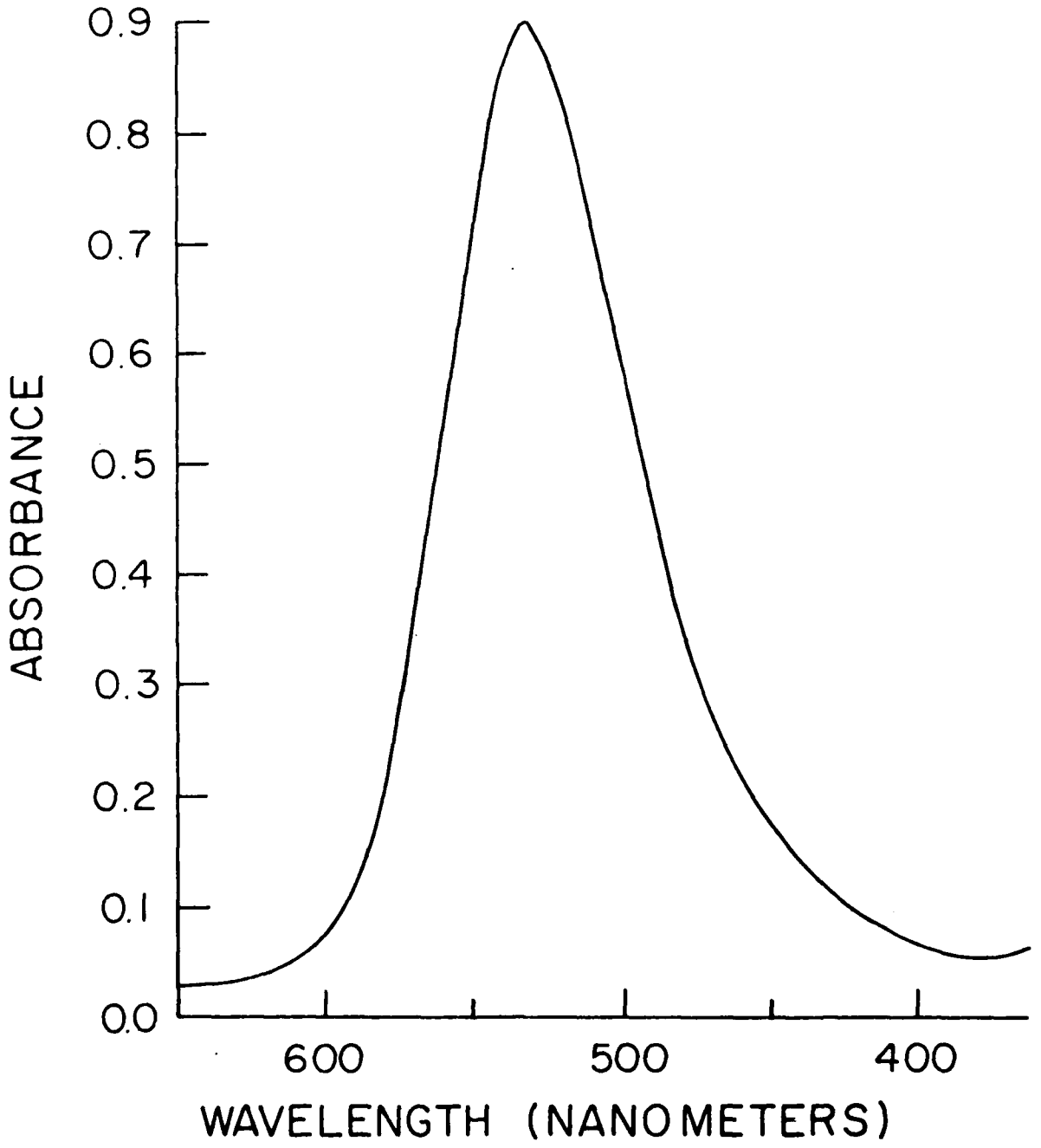
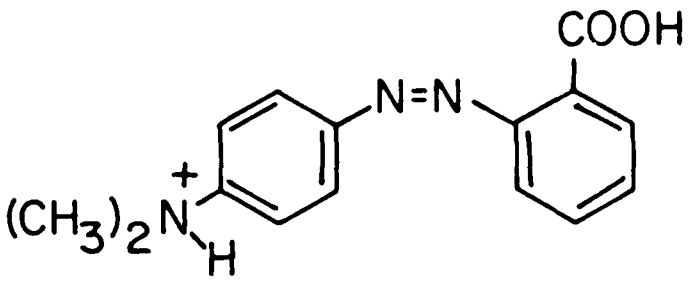


Figure 1. Spectrum of Neutral Red Dyed Nafion 110 Film



ACID CATION

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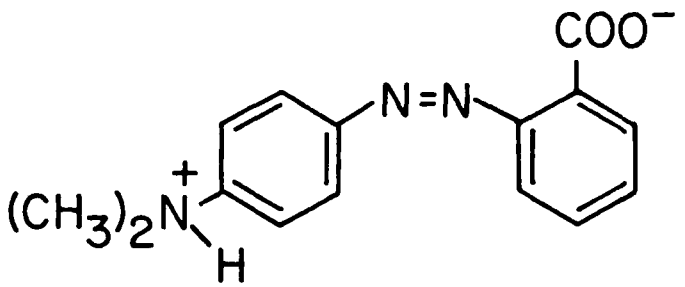

ZWITTERION

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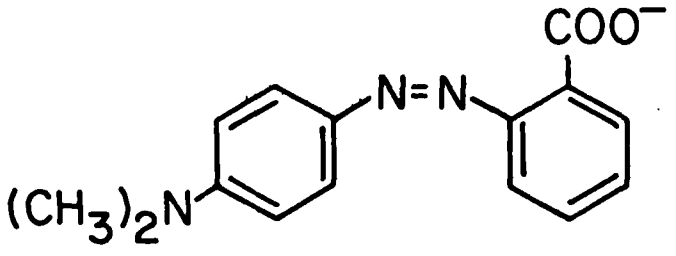

BASE ANION

Figure 2. Methyl Red Equilibria

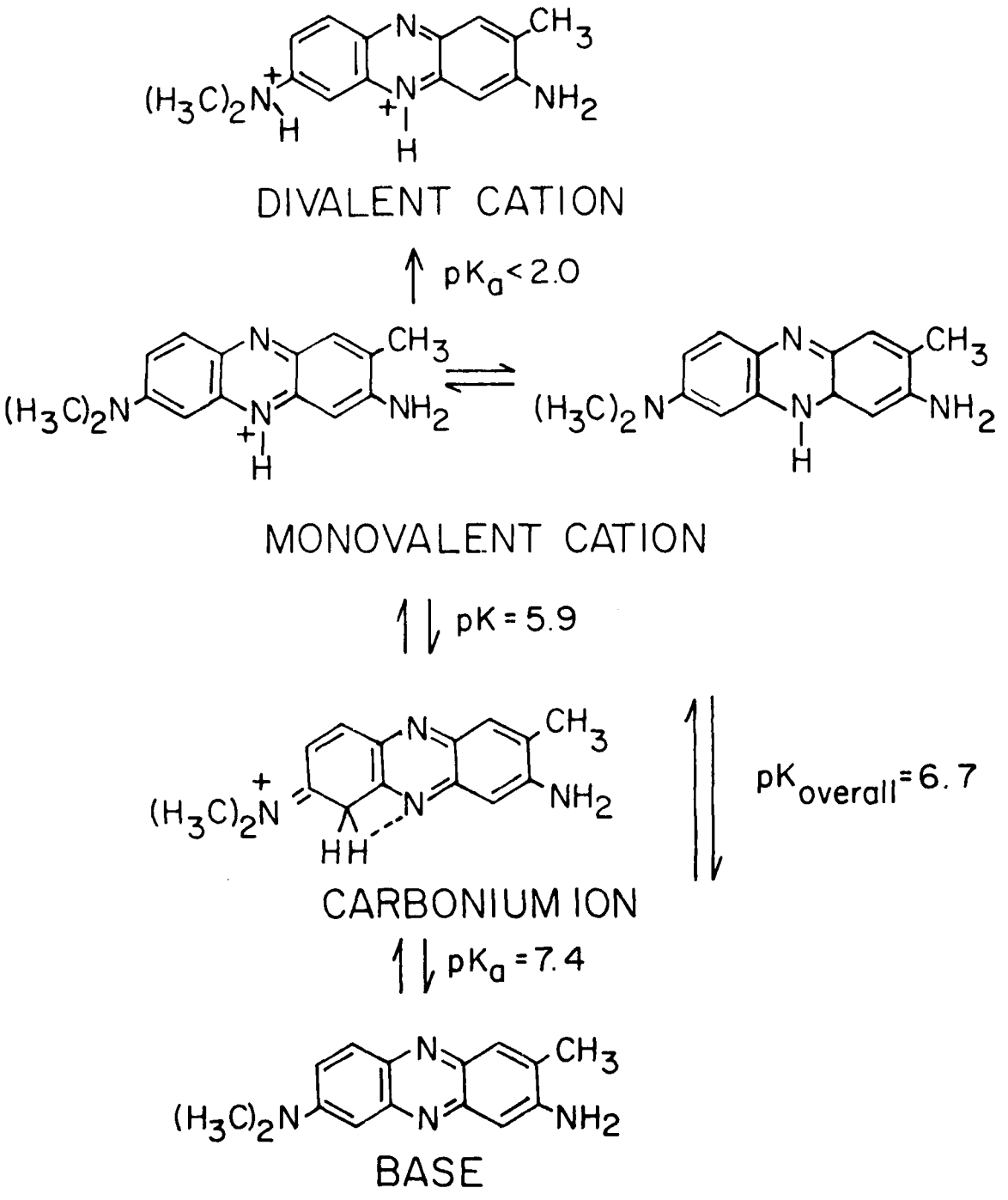

Figure 3. Neutral Red Equilibria

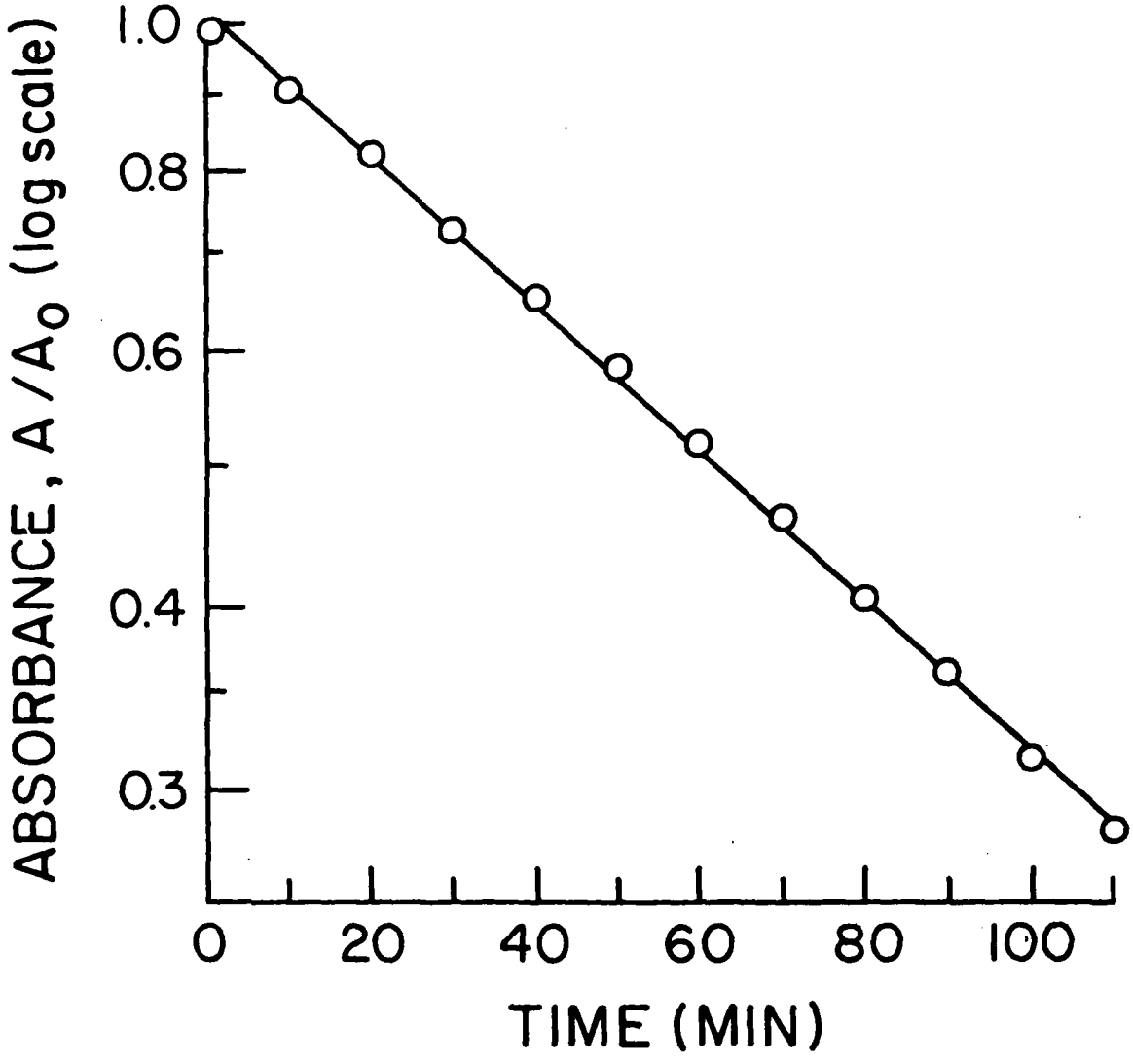

Figure 4. First Order Bleaching Rate of Methyl Red in Nafion 114 with 1.85 mg Cl_2/L at pH 5 and 25°C

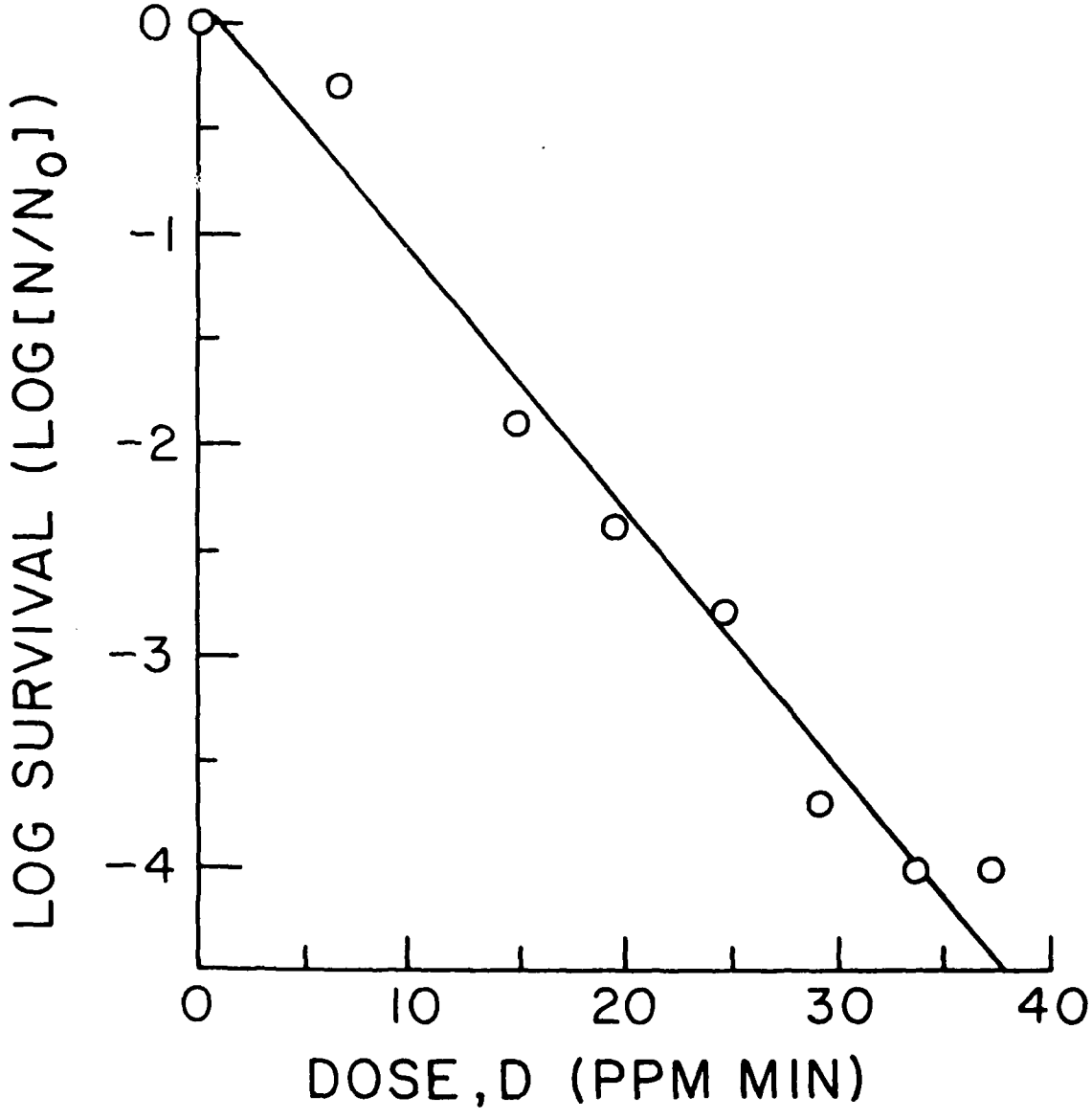

Figure 5. First Order Inactivation of B. subtilis Spores with Dose, D for Experiment 12, Table 9

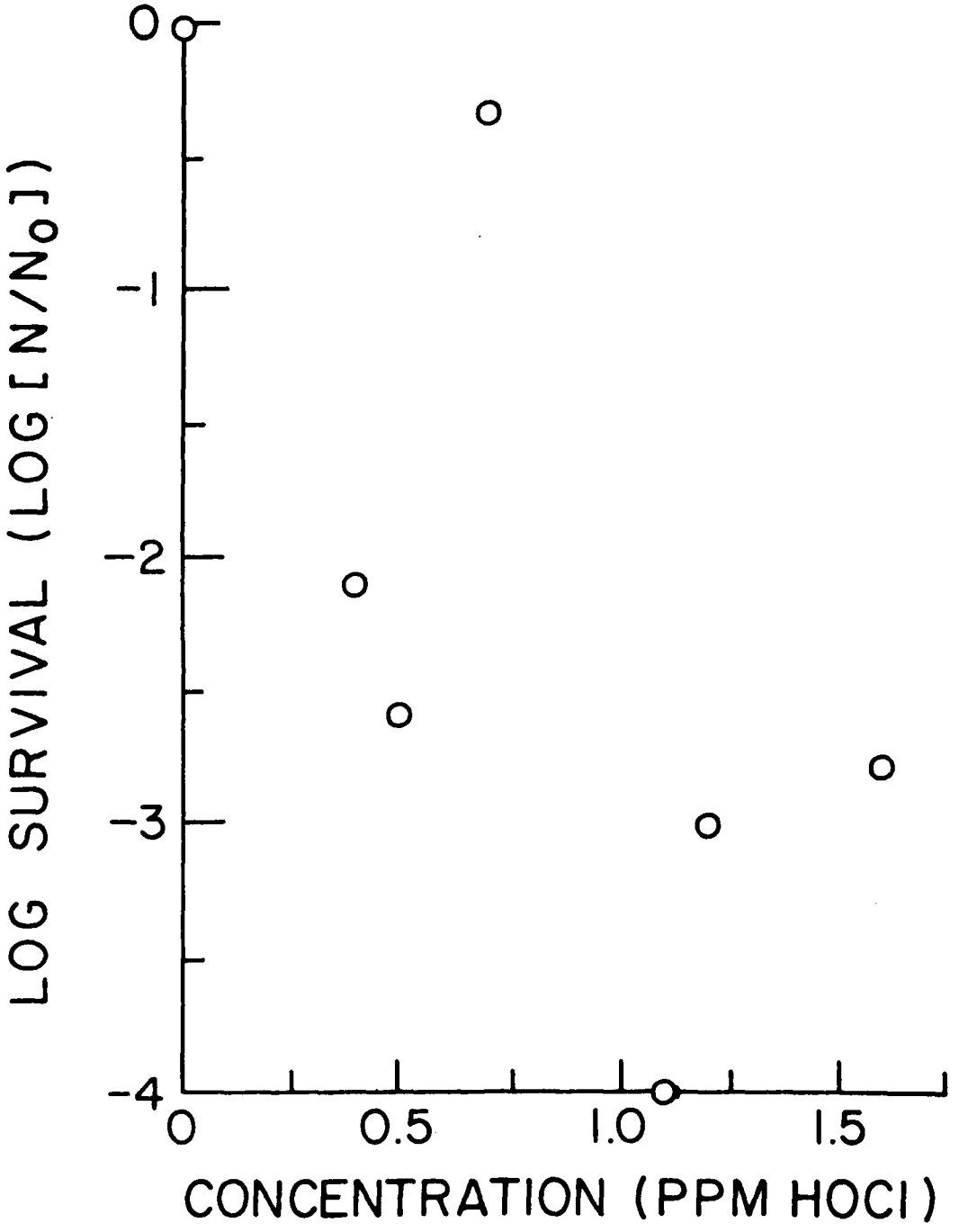

Figure 6. Spore Survival at 15 Minutes vs. HOCl Residual for Experiments 6, 11-14, Table 9

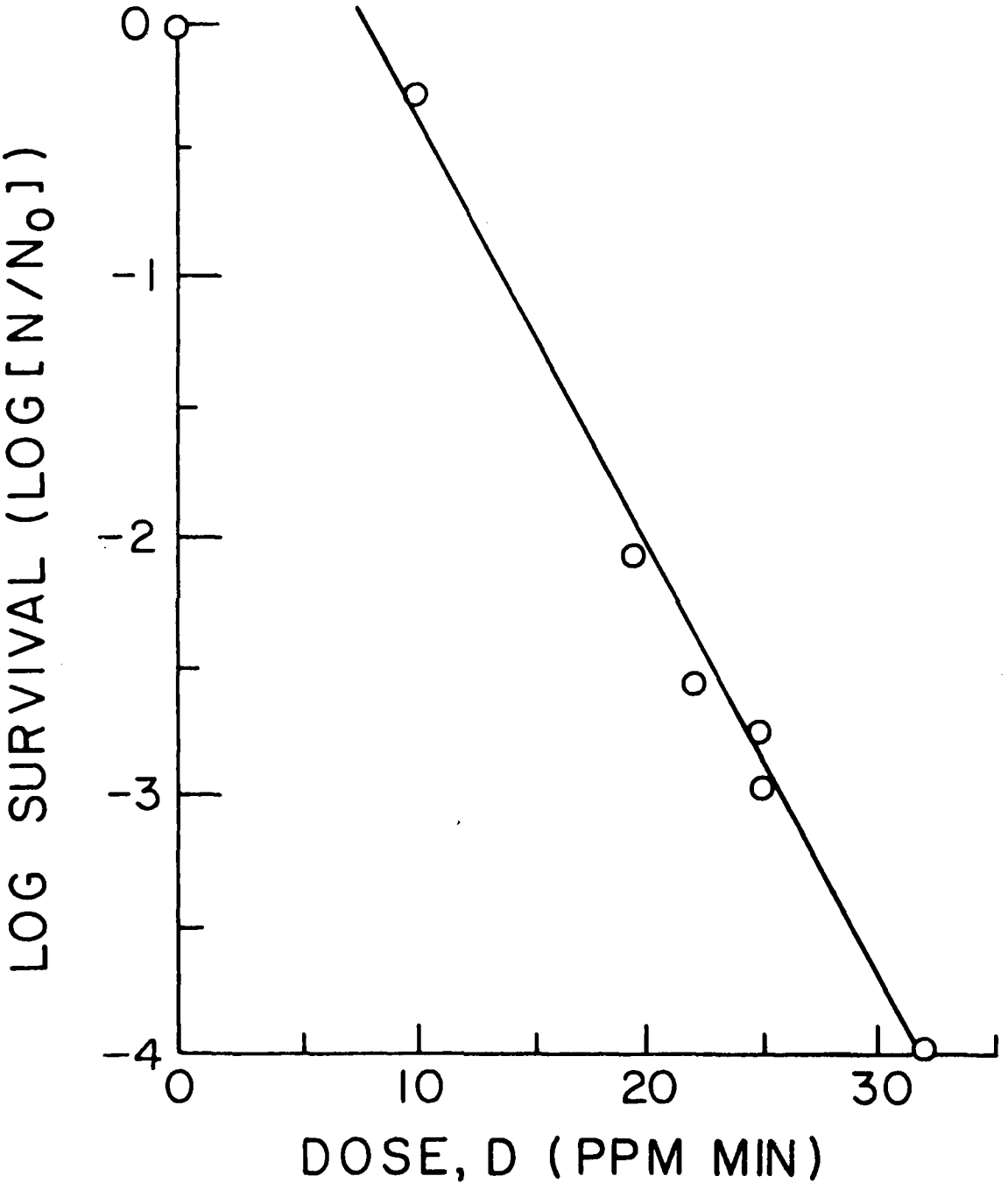

Figure 7. Spore Survival at 15 Minutes vs. Dose, D for Experiments 6, 11-14, Table 9

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